24 Multicenter validation study of a novel StripAssay for cystic fibrosis

H. Puehringer[1], M. Macek Jr.[2], A. Stambergova[2], L. Dvorakova[2], M. Dunø[3], B. Tuemmler[4], S. Hedtfeld[4], M. Tzetis[5], M. Poulou[5], C. Oberkanins[1]. *[1]ViennaLab Diagnostics GmbH, Vienna, Austria; [2]Charles University, Department of Biology and Medical Genetics, Prague, Czech Republic; [3]University Hospital, Department of Clinical Genetics, Copenhagen, Denmark; [4]Hannover Medical School, Department of Pediatrics, Hannover, Germany; [5]National Kapodistrian University, Department of Medical Genetics, Athens, Greece*

Cystic fibrosis (CF), with an incidence of approximately 1 in 3000 live births in Caucasians, is caused by mutations in the cystic-fibrosis transmembrane conductance regulator (CFTR) gene. To date, more than 1500 CFTR mutations have been described, the majority being very rare or private. Worldwide, the most frequent mutation F508del accounts for 30–72% of CF chromosomes depending upon ethnicity. Overall there is great heterogeneity in the remaining pathogenic mutations, as type and distribution vary substantially between populations.
We have developed a reverse-hybridization assay for the rapid and simultaneous analysis of 23 CFTR mutations recommended by the ACMG plus 10 additional ones prevalent in different parts of Europe, as well as the IVS8 polyT (5T/7T/9T) variants. The CF StripAssay, showing a coverage of 70–93% almost all over Europe, is rapid, simple and accessible to automation. The test requires very small amounts of samples, which is of particular importance for prenatal diagnosis and newborn screening. In the first phase of a multicenter validation study genotyping results from 96 out of 111 (86.5%) previously tested samples were confirmed by the CF StripAssay. The remaining discrepant genotypes were due to missing mutant probes on the teststrips. Currently the assay is validated prospectively under routine diagnostic settings in the participating centers.

25 Launch of Luminex xTAG 71 mutations v2 for CFTR screening in Europe

X. Pepermans[1], A. Bosmans[1], M. Philippe[1], T. Leal[1]. *[1]Cliniques Universitaires Saint-Luc, Center for Human Genetics, Bruxelles, Belgium*

Introduction: The potential advantages of Luminex xTAG 71 mutations v2 test, in replacement to the INNO-LiPA CFTR19 + CFTR17+Tn kit, for routine *CFTR* screening were evaluated.
Results: 1350 DNA samples were processed for *CFTR* diagnosis at our university lab. Samples were recruited from the fertility program, the paediatric pulmonology and gastro-intestinal departments. Globally, 114 samples (8.4%) displayed at least one *CFTR* causing mutation. Of these, 5 distinct mutations (L260W; R75Q; Y1092X; R347H; 3120+1G>A) could exclusively be picked up by Luminex; they were confirmed by direct sequencing. L206W was detected from two subjects: in one of them, suffering from idiopathic pancreatitis, S1235R was detected by full exon sequencing; no additional mutation was found in the other subject. R75Q/R75Q was associated with pancreatitis. Y1092X was identified in a classical CF patient also displaying F508del. R347H was found at a heterozygous status during a preconception screening. 3120+1G>A was displayed in a neonate with elevated IRT for whom clinical investigation is currently ongoing at our CF centre. Analyses of the 1350 samples showed 5 polymorphisms: 4 × F508C and 1 × I506V.
Conclusion: The launch of the Luminex xTAG 71 mutations v2 test appears to be extremely valuable for European cities with increasingly heterogeneous and distinct ethnic populations. It covers more gene mutation coverage than any other currently available routine test (71 vs 35 mutations for Luminex vs Innogenetics). An increased (by 0.5%) detection rate, but similar cost, was revealed for Luminex which also accurately detects the three I506V, I507V, F508C polymorphisms recommended by the ACMG/ACOG.

26 Establishment of borderline sweat chloride concentrations: statistical analysis of long-term Czech data

V. Vavrova[1], J. Srajer[2], D. Zemkova[1], M. Macek Jr.[3]. *[1]Charles University 2nd Faculty of Medicine, Pediatric Clinic, Prague, Czech Republic; [2]Charles University 2nd Faculty of Medicine, Prague CF Centre, Prague, Czech Republic; [3]Charles University 2nd Faculty of Medicine, Department of Biology and Medical Genetics, Prague, Czech Republic*

For more than half a century sweat testing has been the "gold standard" for the diagnosis of CF. However, normal and borderline sweat chloride values are not unanimously accepted in different parts of the world. In our country sweat testing has been performed since 1961 and currently we have an experience with more than 35,000 tests, which were performed at a single facility. In this respect we analyzed sweat chloride concentration of 4,974 controls versus 252 CF patients. Sweating was stimulated by pilocarpine iontophoresis, collected in a chloride free filter paper and sweat chloride determination has been performed in a standardized way using coulometric titration by means of *Chloride-Titrator*™ CMT 10. The entire procedure was performed by one qualified technician. For statistical analysis we used chi^2 test and the Bayes' method. The decomposition of our files was logarithmic. Frequentional curves of both groups were constructed. In order to determine the limit between normal and borderline values we used three statistical methods: classical method minimizing the overlap area of both frequentional curves, Bay-max method which maximized the right decisions and Bay-min that minimized incorrect decisions. The limit values of the three methods were 28.48–30.12–31.5 mmol/L, respectively. Only 3.15% of controls exceeded the value of 31.5 mmol/L and in less than 1% their chloride concentration was higher than 40 mmol/L. Therefore, since 1985 all values higher than 30 mmol/L are considered as being "borderline" in our country. We will present our diagnostic strategies and include recent confirmatory results.
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27 Who wants sweat tests and why?

J. Hammermann[1], A. Böhm[1], K. Marx[1]. *[1]University Childrens Hospital, TU Dresden, CF-Center, Dresden, Germany*

Background: Sweat testing is still the standard diagnostic method for detection of cystic fibrosis. The indication for sweat testing ranges from pulmonary, gastrointestinal or other more or less severe clinical findings to routine testing in newborn screening programs. Aim of this investigation is to show, which cause led to sweat testing in the last six years at our hospital and what was the outcome in addiction to the cause of investigation.
Methods: Iontophoresis was done by Wescor MACRODUCT Modell 3700-SYS for iontophoresis and sweat collection. Measurement of chloride concentration in the sweat was done with the Kreienbaum Chloridmeter, measurement of NaCl-Equivalent with Wescor Sweat Check.
Results: From 2006 to 2010 we did over 1000 sweat tests. In about 60% of cases indication for testing was set from our own hospital or outpatient department. A small number of cases was sent from other hospitals of our University like adult medicine, ENT or psychiatry. In 10 to 15% of cases abnormal findings in cystic fibrosis newborn screening caused the sweat test investigation. Gastroenteral or pulmonary led to sweat testing in nearly the same number of cases. In 20 children and one adult the diagnose of cystic fibrosis was confirmed. In 17 of the 20 children abnormal findings in cystic fibrosis newborn screening caused the sweat testing.
Conclusion: Newborn screening is a good indicator for cystic fibrosis independent of clinical symptoms. Nevertheless patients are still sent for sweat testing due to clinical symptoms indicating cystic fibrosis, so doctors are still aware of them.